

# Glucose and Xylose Utilization by *Bacillus Cereus* AS1 During Anaerobic Fermentation

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The aim was to determine the growth potential of *Bacillus cereus* using glucose and xylose as the substrates. In this study a bacterial isolate was identified by 16s rRNA gene sequencing. *Bacillus.cereus* can metabolise glucose and xylose as the sole carbon sources during anaerobic fermentation. The biomass growth profile showed a lag of about 8 h, followed by an increase in the biomass. The growth profile was better on glucose than xylose. The maximum biomass concentration achieved was 3.64 g/L and 2.62 g/L using glucose and xylose, respectively. The higher the initial substrate concentration the higher the growth of the bacteria. The growth of *B. cereus* was accompanied by a decrease in the ORP. Its ability to metabolise xylose proves that it has the potential to convert biomass-derived feed for possible applications as a bio-fertilizer.

## 1. Introduction

*Bacillus cereus* is a rod-shaped, endospore-forming, facultative anaerobic bacteria that is mostly found in soil (Bursová et al., 2018, Luo et al., 2007). It is recognized as one of the major food-borne pathogenic bacteria that causes emetic and diarrheal food-poisoning symptoms (Wong and Chen, 1988). Its tolerance to environmental stressors is high because it forms spores that enable it survive in harsh environmental conditions such as pH (Ahmed et al., 2023), high pressures and extreme temperatures (Soria and Audisio, 2014). The non-pathogenic strains of *B. cereus* have been proven to be able to promote the growth of plants by increasing the shoot and root lengths of plants as well as increasing the seed germination percentage (Aktar et al., 2021). It is able to form biofilms which increases the plants resistance to chemicals heat, UV radiation and other environmental stresses (Kulkova et al., 2023, Kwon et al., 2017). It and can also be used as a biological control agent in the agricultural industry because of its ability to help plants fight off certain bacterial pathogens (Banerjee et al., 2018). The excessive use of fertilizers may cause agricultural pollution via leaching, runoff and erosion, which damages the soils physical, chemical and microbiological characteristics (Cardoso et al., 2021) and in turn can have an impact on the water quality. This makes this microorganism an environmentally friendly alternative to the chemically harsh fertilizers and pesticides that are currently used in the industry.

Nutritional factors, such as the carbon and nitrogen sources in the growth medium have a significant influence the growth and development of bacteria. Optimizing these parameters will enhance the production of *B. cereus*. Zigha et al. (2006) and Zhou et al. (2023) demonstrated that glucose can regulate the growth of *B. cereus*. However, glucose is expensive and using it in the industrial fermentation industry could potentially compete with the global food industry because it is derived from corn-starch. The use of agricultural wastes as sugar substrates for microbial growth could reduce fermentation production costs because they are abundant and inexpensive. Xylose is a major carbohydrate in hemicellulose found in woody or grassy biomass (Bradfield & Nicol, 2016). *B. cereus* can metabolize a number of carbon sources such as glycerol (Kwon et al., 2017), and a variety of dairy products (Ellouze et al., 2021).

The ORP (oxidoreduction potential) is a function of the pH, temperature, amount of dissolved oxygen present as well as the amount of carbon dissolved in the medium (Husson et al., 2006). The ORP of the culture medium affects the growth capacity of microorganisms (Husson et al., 2006) and it has been used to study the carbon and energy flux in several microorganisms.

Hamitouche et al. (2021) found that a decrease in ORP, in anoxic conditions, favoured the growth of *B. cereus*. However, according to Clair et al. (2012) a change in the initial ORP conditions did not affect the growth rate and metabolic activity of *B. cereus*, which indicates that this microorganism can maintain its intracellular redox state by responding effectively to reductive stress.

Another critical factor that affects bacterial growth is the pH of the fermentation medium as influences the cellular metabolism of the microorganism (Wang et al., 2012). Okanlawon et al. (2010) and Yasin and Al-Mayaly (2021) showed that the growth *B. cereus* was at near neutral pH. Metabolic analysis of *B. cereus* fermentation by Le Lay et al. (2015) showed that low pH modifies fermentative metabolism significantly by changing its by-product formation. Low redox potential in combination with low pH resulted in a decrease in the final biomass concentration compared to a low redox potential and neutral pH.

The current study will seek to evaluate the growth potential of *B. cereus* on glucose and xylose using  $\text{MgCO}_3$  as a neutralizing agent.

## 2. Materials and methods

### 2.1 Microorganism and growth medium

*Lactobacillus cereus* strain AS1 was isolated in the laboratory and identified using 16s rRNA sequencing. This strain was used for all the fermentations. Stock cultures (1.5 mL) are stored at  $-40\text{ }^\circ\text{C}$  in 66 % v/v glycerol solutions. Inoculum was prepared by transferring a stock culture to 15 mL of sterilised tryptone soy broth at 30 g/L and incubating at  $37\text{ }^\circ\text{C}$  and 150 rpm for 16 to 24 h.

### 2.2 Fermentation media

The fermentation medium was a replica of the medium developed by Bradfield and Nicol (2014). All chemicals were obtained from Merck KGaA (Darmstadt, Germany) unless otherwise stated. The medium consists of three parts: the nutrient and salt solution, a phosphate buffer, and the glucose/substrate solution. The nutrient and salts solution were composed of 6.0 g/L yeast extract, 10.0 g/L clarified corn steep liquor (Sigma-Aldrich, St Louis, USA), 0.5 g/L NaCl, 0.2  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.2 g/L  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 1.0 mL/L of Antifoam SE-15 (Sigma-Aldrich, Germany). The phosphate buffer consisted of 1.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.8 g/L  $\text{K}_2\text{HPO}_4$ . The D-glucose (Futaste Pharmaceutical Co. Ltd, Shandong, China) and D-xylose concentrations were 70 and 80 g/L.  $\text{MgCO}_3$  acted as the pH regulator and the final concentration in the fermenter was 40 g/L.

### 2.3 Experimental setup and operation

The three feed solutions were prepared in different bottles and diluted with distilled water until a 250 mL solution was obtained. The initial fermenter broth volume was 750 mL. All three bottles were connected in series with silicone tubing to a 1 L fermenter. The silicone tubing was clamped shut to prevent mixing and unwanted reactions from occurring during autoclaving and were separately sterilised by autoclaving at  $121\text{ }^\circ\text{C}$  for 60 min. Once they had cooled down, the three solutions were added to the main reactor, aseptically.

The fermenter was placed on a hotplate stirrer and attached to a temperature measuring electrode. Temperature and stirring speed were controlled at a temperature of  $37\text{ }^\circ\text{C}$  and 300 rpm, respectively. Inoculum was added into the reactor under sterile conditions and anaerobic batch fermentations were carried out for a total of 48 hours. A sample was taken from the onset of the batch fermentation to note initial conditions for a batch run. Samples are taken every 2 hours for the first 12 hours and then the sampling time was increased.

### 2.4 Analytical methods

Each 4 mL sample was diluted with a 1 mol/L HCl solution to break down the carbonate solids that formed inside the reactor. The concentration of glucose (Glc) and xylose (Xyl) in the fermenter broth was determined by High-Performance Liquid Chromatography (HPLC). An Agilent 1260 Infinity HPLC (Agilent Technologies, USA), equipped with an RI detector and a 300 mm  $\times$  7.8 mm Aminex HPX-87H ion exchange column (Bio-Rad Laboratories, USA) was used. Two mobile phases were used for two methods of analysis. The method used a 20 mM  $\text{H}_2\text{SO}_4$  mobile phase solution fed at a flowrate of  $0.6\text{ mL min}^{-1}$ . The HPLC operating conditions resemble the conditions stated in Mokwatlo et al. (2020).

The biomass concentration ( $X$ ) in the reactor was assessed by performing absorbance readings using an Agilent Cary 60 UV-Visible Spectrophotometer (Agilent Technologies, USA) at a wavelength of 600 nm. The oxidation reduction potential (ORP) of the product stream was measured using an Ezdo PL-700 ALS probe (Ezdo, Taiwan).

## 2.5 Microorganism characterization and identification

The phylogenetic analysis was used to identify the bacterial strain. A BLAST program was used to analyze the bacterial stain by comparing it to the 16s rRNA sequence against the NCBI database.

## 3. Results and discussions

Based on 16 rRNA sequences, a phylogenetic tree of the AS1 strain was developed and represented in Figure 1. This strain was identified as a member of *Bacillus cereus*.

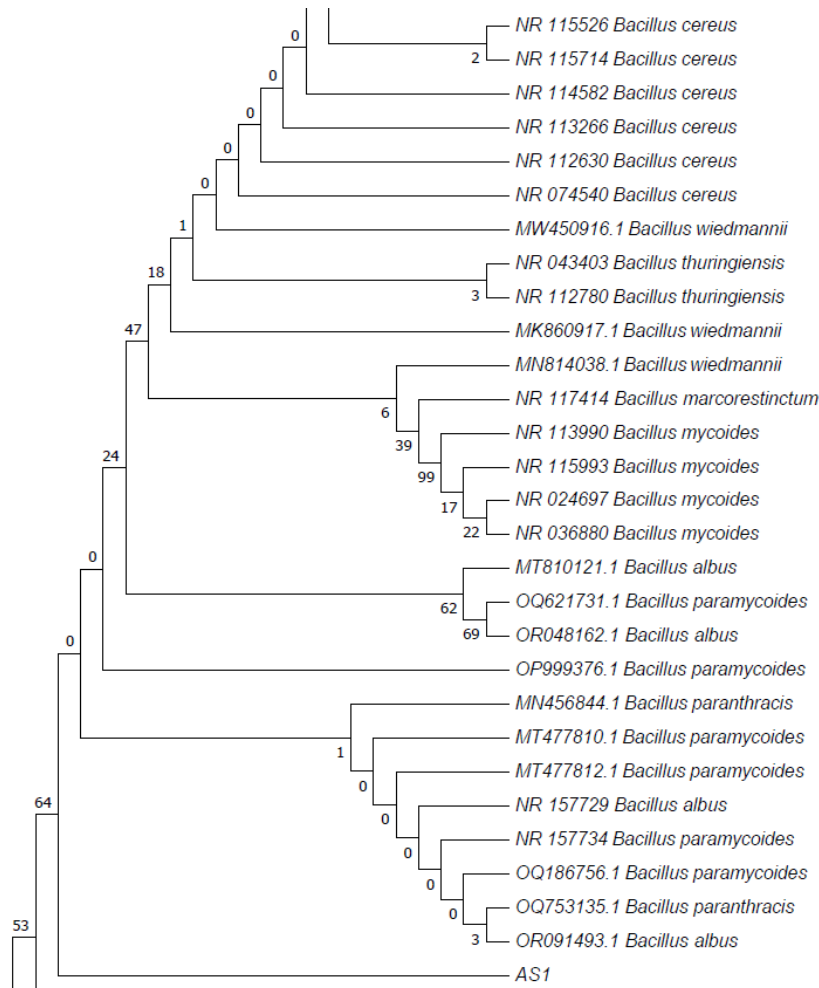


Figure 1: The phylogenetic tree of *Bacillus cereus* strain AS1

The growth rate profile shows that *Bacillus cereus* AS1 can utilize glucose and xylose as carbon sources for its metabolic functions represented in Figure 2a and Figure 2b. The growth rate profile is characterized by a lag phase of approximately 8 h, then a gradual increase thereafter. The cell growth also improved at higher initial glucose and xylose concentrations. At a higher, initial glucose concentration, the bacterial growth rapidly increases, whereas there is no rapid growth on xylose as a the sole carbon source. The maximum biomass concentration on glucose was 3.64 g/L and 2.62 g/L for xylose at an initial substrate concentration of 80 g/L. After 48 h, 69 % and 40 % of the substrate was consumed for glucose and xylose, respectively. An increase in substrate consumption, relates to an increase in biomass formation in the fermenter.

A study on yeasts has found that xylose fermentations are slower than glucose fermentations, because the microorganism might have an insufficient capacity in its metabolism for xylose utilisation (Johansson & Hahn-Hägerdal, 2002), which could also be the reason for *B. cereus*. This data validates the findings in Zhang et al. (2015), that glucose is a superior carbon- source to *B. cereus* AS1 than xylose its consumption rate is higher and because the growth rate was higher using glucose than xylose.

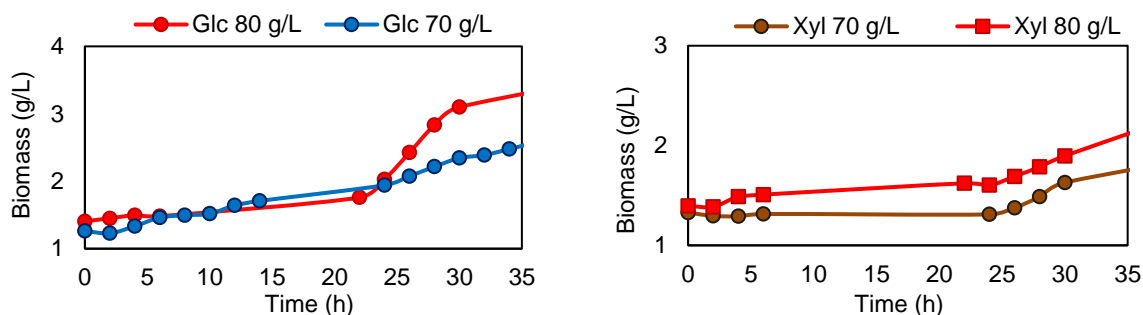


Figure 2: Bacterial growth of *B. cereus* AS1 in a) glucose and b) xylose

Figure 3 represents the effect of the ORP in the fermentation medium on the growth of the bacteria. Some studies have concluded that every microorganism has a preferable redox potential range, within which cell growth is possible. In this study, the decrease in the ORP facilitated the growth of *B. cereus* AS1. The point of the steepest decrease in ORP, occurs at greatest biomass increase. This could imply that the carbon is being used for bacterial growth.

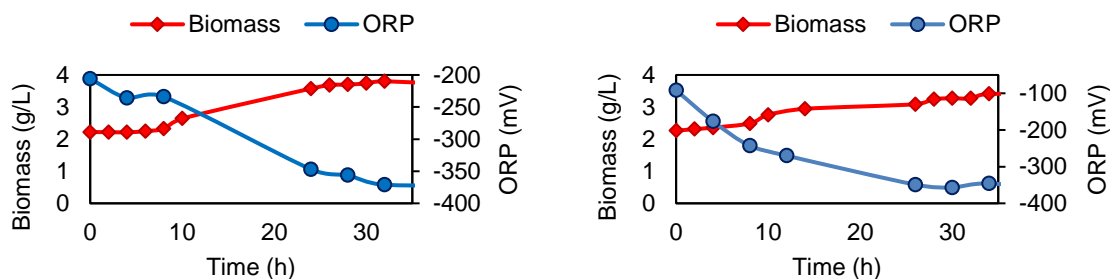


Figure 3: The ORP and bacterial growth of *B. cereus* in a) glucose and b) xylose at 80 g/L.

Table 1 shows the maximum biomass growth and the change in ORP for the duration of the fermentation for Glc as well as Xyl. A low medium ORP signifies a higher availability of electrons (Tan et al., 2014), which could improve the production of biomass thereby promoting an increased production of biomass. There is only a small difference in the change of ORP using glucose and xylose.

Table 1: Summary of the initial substrate concentrations, biomass concentrations and ORP

Substrate/Product	Initial concentration (g/L)	Final biomass concentration(g/L)	Change in ORP (mV)
Glucose	80.0	3.64	-166
Xylose	80.0	2.62	-173

#### 4. Conclusions

In this study, the bacteria isolated from the laboratory was identified to be *Bacillus cereus* by phylogenetic analysis. It has the ability to catabolise glucose as well as xylose as the sole carbon source under anaerobic, batch fermentation. This implies that *B. cereus* AS1 has the potential to be cultivated using plant-based renewable resources. Lower ORP conditions favoured the microbial growth of *B. cereus* AS1. A model to predict the growth kinetics of *Bacillus cereus* AS1 on glucose and xylose must be developed.

## Nomenclature

Glc – Glucose

Xyl – Xylose

ORP – Oxidation reduction potential

X – Active biomass

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